Transcription: **Gene control by targeted histone acetylation**

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A transcriptional regulator in yeast, Gcn5p, activates transcription by targeted acetylation of specific lysine residues in the amino-terminal tails of histones. This targeted modification is restricted to nucleosomes assembled on the promoters of Gcn5p-responsive genes.

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The recent discovery that transcriptional activators have histone acetyltransferase activity stimulated renewed attention on the role of chromatin structure in gene regulation [1]. The modification of histones *in vitro* does not, however, prove that these abundant structural proteins are the true targets for transcriptional regulation *in vivo*, or that acetyltransferase activity *per se* has a role in transcriptional regulation. Other components of the transcriptional machinery, such as p53, TFIIE and TFIIF can be acetylated *in vitro* [2,3]. Modification of these more limiting factors in the eukaryotic nucleus might be the main way that acetyltransferases influence transcription. Similarly, activators might influence the recruitment or function of the basal transcriptional machinery by mechanisms independent of acetylation either of the histones or of any other protein [4].

These issues have now been experimentally tested in the budding yeast *Saccharomyces cerevisiae*, using an insightful combination of genetics and biochemistry. The new findings firmly establish the necessity of acetyltransferase activity for the activation of transcription [5,6]. Furthermore, they identify specific lysines in the nucleosomal histones as targets for the acetyltransferase [7], and demonstrate that nucleosomes assembled on the promoters of responsive genes are selectively enriched in acetylated histones [6]. The targeted modification of nucleosomal histones is revealed to be a major contributor to transcriptional activation.

The key molecule in these experiments is the yeast transcriptional activator Gcn5p, the archetypical regulatory histone acetyltransferase [1]. Gcn5p was originally defined as a component of an activator complex that contains two other proteins, Ada2p and Ada3p, and that facilitates the action in yeast of transcription factors with acidic activation domains, such as the endogenous protein Gcn4p and the artificial fusion protein Gal4–VP16 [8,9]. An activator complex containing Gcn5p can potentially include other transcriptional regulators such as Spt7 and Spt20; under these circumstances it is known as the 'SAGA complex', for '*S*pt, *A*da, *G*cn 5 *a*cetyltransferase'. The SAGA complex is involved in the regulation of numerous yeast genes (reviewed in [5]).

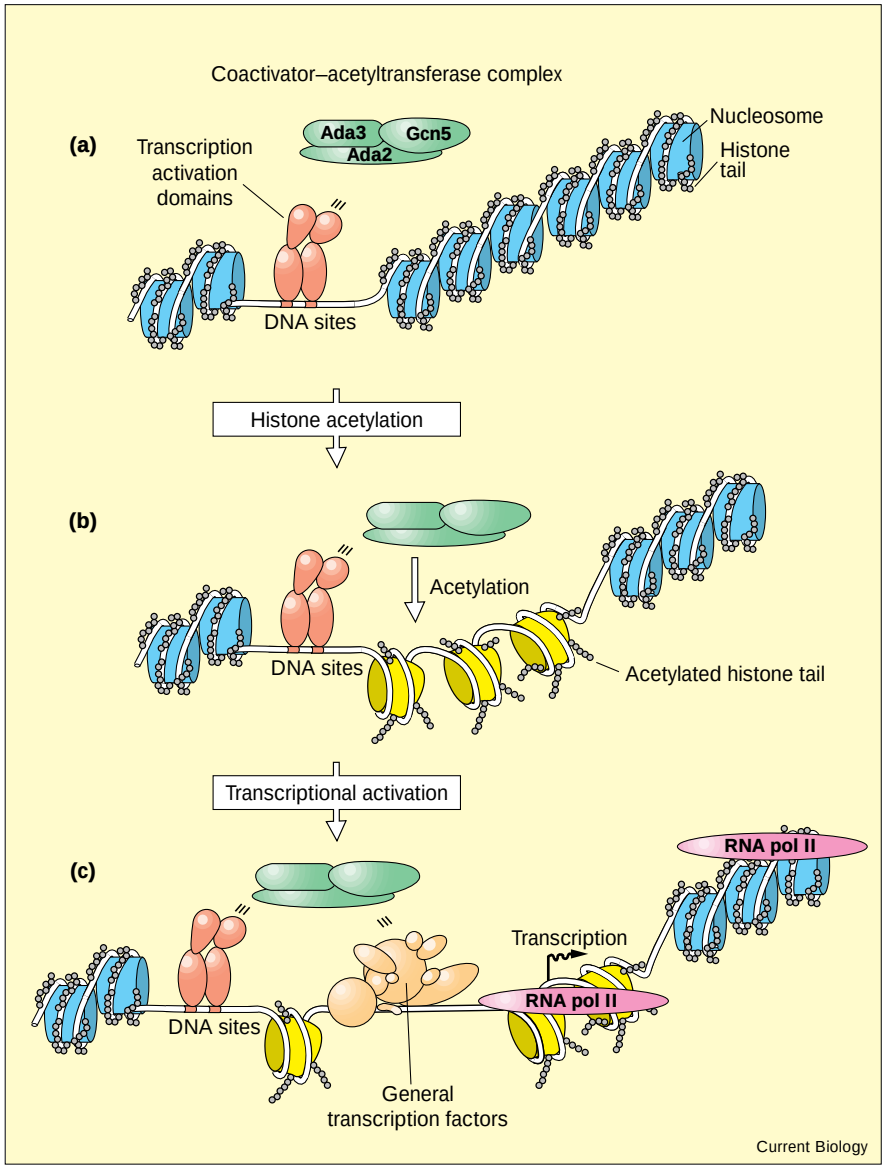
Gcn5p homologs have been identified in species as diverse as *Tetrahymena* and *Homo sapiens*, and the availability of these various sequences suggested possible structural and functional domains within the protein [5,6]. Conserved residues within the histone acetyltransferase domain suggested sites for mutations that might influence enzymatic activity. Alanine-scanning mutations throughout the acetyltransferase domain generated proteins with a 20-fold variation in enzymatic activity, as assayed using purified histones as substrates for acetylation *in vitro*. Indeed, yeast strains containing these mutant *GCN5* genes were found to be impaired in the acetylation of chromatin *in vivo* [5,6]. These basic observations have facilitated investigation of the *in vivo* importance of chromatin acetylation.

Functional Gcn5p is known to be required in yeast for the maximal expression of genes involved in amino acid biosynthesis [8], and strains deficient in Gcn5p acetyltransferase activity are slow to grow under conditions of amino acid starvation [5,6]. The significance of acetyltransferase activity for transcription itself is shown by an excellent correlation between the ability of the various mutants forms of Gcn5p to acetylate free histones and their ability to activate genes in the presence of a Gal4–VP16 fusion protein [5,6]. These experiments strongly suggest that the acetyltransferase activity of Gcn5p is required for the activation of Gcn5p-responsive genes. They do not, however, identify the true targets for acetyltransferase activity.

Evidence that histones really are physiological acetylation targets has come from the work of Kuo *et al.* [6], who have shown that histones are modified in response to recruitment of Gcn5p *in vivo*, and have delimited the zone of histone modification by Gcn5p to the vicinity of the activated promoter itself. Chromatin immunoprecepitation assays using antibodies against acetylated histone H3 revealed a 3–6-fold enrichment of the modified protein within chromatin-containing promoter sequences, relative to chromatin assembled on control sequences that are more than a kilobase away from the promoter.

The acetylation of histone H3 at the Gcn5p-activated promoter occurs coincidently with transcriptional activation. The enrichment in acetylated histones does not

Figure 1



Transcriptional activation by histone acetylation. (a) Sequence-specific DNA-binding transcription factors (orange) target a coactivator–acetyltransferase complex (green) to promoter chromatin which is in a repressive nucleosomal structure (blue). (b) The coactivator–acetyltransferase complex locally modifies promoter chromatin by acetylation of the histone tails, creating a transcriptionally competent nucleosomal structure (yellow). (c) The coactivator –acetyltransferase complex also facilitates recruitment of the basal transcriptional machinery (pink) to the accessible promoter chromatin, and transcription is thus activated. But although RNA polymerase II carries out transcriptional elongation through chromatin throughout the transcription unit, histone acetylation remains selectively enriched in promoter chromatin. Thus transcription itself does not cause the enrichment of acetylated histones; it is acetylation of the histones that causes transcription.

extend throughout the entire transcription unit [6]. It is thus unlikely that the accumulation of acetylated histones in the vicinity of the promoter of a responsive gene is a consequence of the chromatin disruption that occurs following the recruitment of RNA polymerase and initiation of transcription. Instead, these results suggest that the recruitment of Gcn5p to a promoter leads directly to the targeted modification of histones. The local modification of chromatin at the promoter might be a prerequisite for access of the transcriptional machinery to DNA and the subsequent activity of RNA polymerase in a chromatin environment (Figure 1; see [10,11]). Genetic evidence in support of this hypothesis comes from new experiments in which both Gcn5p and the histones themselves were mutagenized [7].

Zhang *et al.* [7] substituted specific lysines in the amino-terminal tails of histones H3 and H4, and examined the consequences for both the growth of yeast cells and the transcription of Gcn5p-responsive genes in yeast strains with and without functional Gcn5p. The experiments were designed to test whether modification of the histones at specific sites is an essential function of Gcn5p. The major target of Gcn5p acetyltransferase activity *in vitro* is lysine 14 in the amino-terminal tail of histone H3. Substitution of lysine 14 by either arginine or glutamine was found to have little functional consequence in strains containing Gcn5p, but in stains that were both mutant for histone H3 and deficient in Gcn5p, cell growth was markedly inhibited.

The acetyltransferases thus appear to show functional redundancy, as histone mutations and Gcn5p deficiency are both required to produce strong growth phenotypes. Moreover, the function of acetylation cannot be that of simply neutralizing the charges on basic lysine residues, as the substitution of lysine by either basic arginine or neutral glutamine leads to similar phenotypes. But although histone H3 is selectively acetylated by Gcn5p *in vitro* [12] and is enriched within Gcn5p-responsive promoter chromatin *in vivo* [6], it is not necessarily the major functional site of histone modification *in vivo*. To address this issue, the functional significance of acetylation of other sites within the amino termini of histone H3 and H4 was also examined [7].

Analysis of histone H4 modification states *in vivo*, in the presence or absence of Gcn5p, showed that four different lysine residues — 5, 8, 12 and 16 — in H4's amino-terminal tail can be modified by the Gcn5p acetyltransferase. Mutagenesis of either lysines 8 and 16, or 5 and 12, led to a marked slowing of yeast cell growth [7,13]. Deficiency of Gcn5p did not alter this growth phenotype when lysines 8 and 16 were replaced with glutamine, but when they were replaced with arginine, it significantly enhanced the slow growth phenotype [7]. Substitution of lysine 5 and 12 by either arginine or glutamine further reduced growth rates in Gcn5p-deficient cells. These results again indicate that the loss of acetylatable lysines has major consequences for chromatin function. The essential role of acetylation of the histone H3 and H4 amino termini is most clearly demonstrated by the fact that simultaneous substitution by arginine of lysine 14 in histone H3, and lysines 8 and 16 in histone H4, led to cell death [7]. A direct connection between acetylation of these three lysines and the Gcn5p-dependent transcriptional response follows from the effects of their substitution by glutamine, which bypasses the Gcn5p requirement for transcriptional activation by Gal4–VP16.

These new experiments show that the acetyltransferase activity of Gcn5p is essential for its ability to activate transcription [5,6]. They further identify specific lysines in histones H3 and H4 that are functional targets of the acetyltransferase [6,7], and indicate that the promoter, and not the transcription unit, is the relevant domain of chromatin modification [6]. The direct connection between targeted chromatin modification and transcriptional control is clear, but the mechanistic details remain obscure. Although the acetylation of lysines within the amino-terminal tail domains of H3 and H4 has been shown to promote transcription [10,11], the mechanism by which this modification leads to an alteration in chromatin structure and transcriptional competence is unknown.

Complete removal of the histone amino-terminal tails, either genetically or biochemically, causes similar yeast cell phenotypes to those observed following the acetylation of tail-domain lysines, or their replacement by glutamine. This suggests that acetylation of the histones destabilises aspects of chromatin structure that are dependent on the presence of the tail domains. Alternatively, components of the transcriptional machinery, such as the RNA polymerase holoenzyme, might recognize domains of chromatin that are identified by a particular combination of modified lysine residues. Determining exactly what the histone tails do to chromatin structure will be necessary to further unlock the mysteries of transcriptional control.

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